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— SECTION B —

CHEMICAL SCIENCES

Contents

	Page
The Determination of Heavy Water—*I. E. Puddington* - - -	1
Production and Properties of 2,3-Butanediol. XXX. Determination of the Fermentation Acids by Partition Chromatography—*A. C. Neish* - - - - - - - - - - -	6
Une Nouvelle Synthèse de l'Acide Aminoadipique—*Roger Gaudry*	21
The Testing of Activated Charcoals by the Isopiestic Method —*H. Sheffer, K. T. Waldock, and J. B. Ferguson* - - - - -	25
n-Eicos-11-enoic Acid—*C. Y. Hopkins, Mary J. Chisholm, and John Harris* - - - - - - - - - - - - - - -	35

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CANADIAN JOURNAL OF RESEARCH

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VOL. 27, SEC. B. JANUARY, 1949 NUMBER 1

THE DETERMINATION OF HEAVY WATER[1]

I. E. PUDDINGTON

Abstract

A reasonably simple procedure for determining the concentration of heavy water solutions, based on the comparison of the vapor pressures of these solutions with pure water, has been developed. The method is useful for samples as small as 0.5 mgm. and the sample may be recovered. Individual determinations require about two hours. In the work reported, mean determinations did not vary from the best curve through the experimental points by more than 0.2 mole % of D_2O, while the spread in any group of readings was about ± 0.25 mole %.

Introduction

Various methods have been proposed for the determination of the concentration of heavy water solutions. Procedures based on the measurement of vapor pressures of the solutions do not appear to have been employed, however. The recent development of a simple mercury manometer, capable of high sensitivity, made this method seem feasible since the published values for pure D_2O indicate a vapor pressure difference from pure water of approximately 0.03 mm. for each mole per cent of D_2O present in solution at 20° C. This communication presents experimental results that show that the method is not only feasible but should constitute a useful analytical tool.

Experimental

The apparatus, shown in Fig. 1, is built around the differential manometer. Although this manometer has been described elsewhere (2) a short description will be repeated here to clarify the experimental procedure.

The short, wide cylinders *A* and *B* form the arms of a U-tube. These are connected with 3 mm. capillary through a stopcock at *C*. The bulb at *D*, which has about one-half the volume of *B*, is connected to *B* with a length of capillary selected for uniformity of bore. The sections of 1 mm. capillary above *B* and *D* are etched at *F* and *G*.

The manometer is operated as follows. Sufficient clean mercury is admitted through *H* to the evacuated assembly, and the end of the thread adjusted to *G*. Stopcock *C* is left open and the three-way tap at *J* turned to connect the spaces

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above the mercury in A and B. The mercury in these two cylinders should now be at the same level. Stopcock C is then closed and air admitted through a slow leak at I; this forces the mercury from D into B filling it to F. The

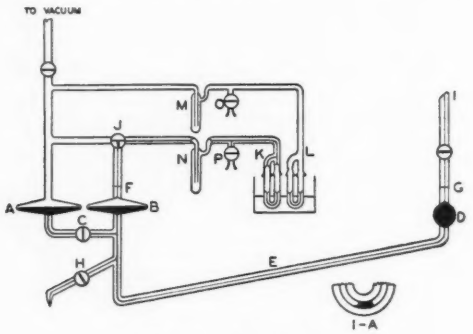

FIG. 1. *Apparatus.*

volume of mercury in the apparatus should be such that on adjustment of its level at F the other end of the thread should be near the lower end of the capillary E. This point on E represents the zero reading of the manometer. The mercury is now returned to G, stopcock C opened and J turned to connect B with K. If the sample holders K and L contain liquids that differ slightly in vapor pressure, with that in L being the higher, mercury will flow from A to B until equilibrium is established. If C is now closed and the procedure for finding the zero point repeated, the volume of mercury that flowed from A to B will be represented by the increased length of the mercury thread in E. The pressure difference may then be calculated from the known geometry of the apparatus.

It is obvious that A and B must be mounted rigidly with respect to each other. In the present work they were cast in a block of plaster of Paris, which was supported on a stand of four 1/2 in. rods clamped to each other and secured to the bench with floor flanges. Stopcock C was supported on the same frame.

The cylinders A and B were made from 46 mm. I.D. Pyrex tubing while E had a diameter of 0.78 mm. This gives a theoretical magnification of pressure differences of 1740. Actually, the diameter of one of the cylinders was slightly reduced in forming the cylinder, and the magnification factor found on calibration with a McLeod gauge, using air as the working substance, was 1650, that is, 1 mm. on the E scale was equivalent to a pressure difference of 0.000606 mm. Cylinder B had a volume of 23 cc. and the inner side walls of both A and B were etched with carborundum powder prior to assembly.

The sample holders K and L were made in the form of U's and were attached to the tubes connected with the manometer arms with standard taper ground

glass joints. The curved portions of these holders, which actually contained the samples, were made from 0.5 mm. capillary. Samples condensed here in slugs of the form shown in Fig. 1-*A*. This procedure ensured surfaces of equal curvature, and any error due to the high vapor pressure of small droplets was avoided. No difficulty was experienced in the bridging of the condensed droplets to form slugs, provided the capillaries were kept clean, and this could be done readily by periodic, thorough steaming. The capillary portions were almost completely immersed in the thermostat liquid.

Test samples of heavy water were made up gravimetrically from a sample of known concentration and water that had been purified by distillation from alkaline permanganate. Amounts of 1 to 2 mgm. were drawn up into short pieces of 0.5 mm. capillary placed in a small container, and attached to the apparatus at *P*. A similar sample of pure distilled water was connected at *O*. These samples were then admitted to the evacuated apparatus and trapped at *M* and *N* using dry ice as a refrigerant. Dissolved air was removed by several cycles of condensation and evaporation, the system being pumped down to a "click" vacuum on a McLeod gauge with each condensation. When free from air, the samples were condensed into the capillaries of the sample holders, and readings of the pressure difference taken after equilibrium was established. The thermostat was held at $20 \pm 0.01°$ C.

About six readings of the pressure difference were taken for each sample, the zero point being checked after each reading. This procedure is desirable, since changes in room temperature will change the zero reading of the manometer. The zero point could be checked without dilution of the D_2O solution by forcing the mercury nearly to *J* in the capillary above *B* before the three-way stopcock was turned to connect *A* with *B*. A similar procedure was followed before connecting *B* with *K* once again. Equilibrium was established rapidly and it was quite possible to take readings every 15 min.

Although samples of not less than 1 mgm. were used in the present work, it seems quite probable that these could be reduced to 0.5 mgm. by reducing the dead space. The volume of *B* may be made as small as 14 cc. without sacrificing the 46 mm. diameter. By making the trap *N* small and using large diameter capillary for connecting tubing, about 0.3 mgm. of vapor at 20° C. should fill the dead space, leaving a slug of liquid about 1 mm. long in the sample holder. Very little fractionation of the sample takes place and the use of such small samples seems to be quite feasible.

Some idea of the precision of the method can be obtained from the following set of *E* scale readings obtained for one sample. These figures were selected at random and are representative of the reproducibility obtainable: 219, 224, 213, 216, 203, 214. Examination of Fig. 2 shows that these figures correspond to a total spread of 4.75 to 5.25 mole % of D_2O.

The experimental values for the vapor pressure lowerings, using the micro samples, are given in Table I.

TABLE I

EXPERIMENTAL VALUES OF VAPOR PRESSURE LOWERING

Mole % D_2O	Mean E scale reading, mm.	ΔP, mm.
1.42	60	0.036
3.38	145	.088
4.86	215	.130
9.89	438	.265
13.15	575	.348

These values, together with results obtained when large samples (0.5 cc.) were used, are shown graphically in Fig. 2. With the large samples, bulbs equipped with magnetic stirring devices were used as sample holders. The

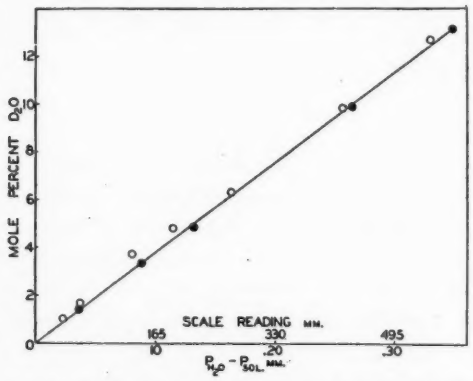

FIG. 2. *Lowering of the vapor pressure with increased D_2O concentration. Open circles, values obtained with 0.5 gm. samples; filled circles, values obtained with micro samples.*

data from the large samples are more erratic than those from the micro samples. They show, however, that fractionation of the sample during a determination is not serious since in no instance do they deviate from the micro samples by more than 0.5%. None of the mean values for the micro samples depart from the best curve drawn through the points by more than 0.2%. The microtechnique has the added advantage of requiring much less time for degassing the sample.

Since H_2O and D_2O might be expected to form an ideal solution and obey Raoult's law, a reasonable value for $P_{H_2O} - P_{D_2O}$ should be obtained by extrapolating the curve in Fig. 2 to 100 mole % of D_2O. The value found on doing this is 2.62 mm. This is probably slightly high owing to sample

fractionation. Extrapolation of the best curve through the points obtained with 0.5 gm. samples indicates a value of 2.47 mm., which is in good agreement with the value of 2.48 mm. given by Miles and Menzies (1).

Measurements of ΔP at concentrations higher than 14 mole % of D_2O were not attempted, since the total range of the manometer used in the work was only 0.4 mm. The work done, however, indicates the usefulness of the method and probably covers the range that will be encountered most frequently. Higher concentrations could, of course, be determined by suitably diluting the sample.

Although no specific examples can be cited, it is suggested that this approach might be of use in determining the composition of other two-component systems.

Acknowledgment

Grateful acknowledgment is made to Dr. E. W. R. Steacie who suggested the problem.

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2. Puddington, I. E. Rev. Sci. Instruments, 19 : 577. 1948.

PRODUCTION AND PROPERTIES OF 2,3-BUTANEDIOL

XXX. DETERMINATION OF THE FERMENTATION ACIDS BY PARTITION CHROMATOGRAPHY[1]

By A. C. Neish[2]

Abstract

A procedure has been developed for the estimation of butyric, acetic, formic, succinic, and lactic acids in fermentation solutions. The acids are extracted by ether, separated by partition chromatography on a column of silica gel using benzene–butanol mixtures, and each determined quantitatively by titration with 0.01 *N* sodium hydroxide using phenol red as indicator. Recoveries are quantitative and determinations accurate within ± 2%. A method is also given for the quantitative separation and estimation of butyric, propionic, acetic, and formic acids, as well as a modified procedure for preparation of silica suitable for partition chromatography.

Introduction

Bacteria known to ferment glucose to 2,3-butanediol all form organic acids as by-products, sometimes in quite high yields. Those acids most likely to be found are *n*-butyric, acetic, formic, succinic, and lactic. Until recently these were determined in this laboratory by the old established methods based on rate of steam distillation, oxidation reactions, and solubility of the salts (10). These methods have been replaced by a procedure based on the separation of these acids by partition chromatography on silica which is both more rapid and accurate when a variety of acids are to be determined. The purpose of the present paper is to describe this procedure in detail.

The technique of partition chromatography on silica gel was first introduced by Martin and Synge (9) who applied it to separation of the acetamino acids. Tristam (14) has extended their work while Elsden (2) and Ramsey and Patterson (12) have applied the method to the separation and identification of the volatile saturated fatty acids (C_3–C_4). Recent work (11, 13) has extended this technique to fatty acid mixtures containing C_5 to C_{10} acids. Isherwood (6) has modified the original method and applied it to the separation and estimation of the organic acids commonly found in fruits. Claborn and Patterson (1) have made use of partition chromatography in determining lactic and succinic acids in foods, but they were unable to separate these acids by chromatography alone. The procedure given below for estimation of the fermentation acids, essentially a modification of Isherwood's method, gives separation of all the fermentation acids mentioned above on one passage through the column.

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Experimental

Reagents

(1) *0.01 N Sodium hydroxide (carbonate-free).*

(2) *0.01 N Hydrochloric acid.*

(3) *0.05% Phenol red solution.*

This was prepared by grinding 0.100 gm. of phenol red in a mortar with 28.5 ml. of 0.01 *N* sodium hydroxide and diluting to 200 ml. with distilled water.

(4) *0.0025% Phenol Red in 0.001 N Sodium Hydroxide.*

This was prepared by mixing 10 ml. of 0.05% phenol red and 20 ml. of 0.01 *N* sodium hydroxide, then diluting to 200 ml. with carbon-dioxide-free distilled water. This solution is used to detect acids in the solvent coming from the column.

(5) *Solvent Mixtures for Developing the Chromatogram*

The best grade of solvents available commercially are used without further purification. All mixtures are made by volume; thus benzene containing 5% of *n*-butanol (BeB_5) is prepared by mixing 5 volumes of *n*-butanol (B) with 95 volumes of benzene (Be). The other mixtures used in the regular procedure are benzene containing 10% *n*-butanol (BeB_{10}) and benzene containing 25% *n*-butanol (BeB_{25}). Before use these mixtures must be equilibrated with the liquid used to wet the silica, in this case 0.01 *N* hydrochloric acid. This is done by shaking 400 ml. of solvent with 80 ml. of 0.01 *N* hydrochloric acid in a one-liter separatory funnel for at least two minutes. After the mixtures have stood for 5–10 min. the aqueous layer is drawn off and discarded and the somewhat turbid benzene phase is filtered through a dry, fast filter paper to remove the remaining droplets of the aqueous phase. The clear, wet solvent is then stored in a 500 ml. Erlenmeyer flask provided with a cork stopper. Mixtures of chloroform and butanol have been quite popular for developing the chromatogram but they are not suitable for separation of succinic and lactic acids.

(6) *Silica Gel Suitable for Partition Chromatography*

The contents of a 1 lb. bottle of reagent grade or "pure" sodium metasilicate ($Na_2SiO_3 \cdot 9H_2O$) is transferred to a 1 liter graduated beaker, using enough distilled water to give a final volume of 620 ml. The beaker is warmed in a steam bath, with occasional stirring until the silicate is dissolved. Usually the solution is somewhat turbid but filtration is unnecessary. The beaker is then placed in a bath containing ice–water and stirred manually from time to time until the temperature falls to 35° C. During this period enough methyl orange powder is added to give the solution a yellow color. The precipitation is then carried out by adding 10 *N* hydrochloric acid slowly

from a separatory funnel as the solution is being stirred vigorously. The temperature is kept in the range 36° to 38° C. by controlling the rate of addition of the acid. The precipitation passes through a thick stage when the mixture has about the same consistency as porridge but it never actually sets to a gel. It is necessary to beat the mixture thoroughly at this stage. The addition of acid is continued until the indicator remains pink after thorough mixing. It requires about 14 min., approximately 250 ml. being added. A further 10 ml. of acid is then added and the beaker allowed to stand at room temperature for three hours with occasional stirring. More acid is added, in 10 ml. portions, if the indicator loses its pink tinge. The silica is filtered off on a Büchner funnel, washed with three 500 ml. portions of distilled water, resuspended in one-liter of 0.2 *N* hydrochloric acid and allowed to age overnight (18 to 20 hr.). It is then washed on the Büchner funnel until the washings are about pH 4.5 as determined with indicator paper. This requires approximately ten 500 ml. portions of distilled water. Finally it is washed with a 500 ml. portion of 95% ethanol followed by 500 ml. of ethyl ether. After drying in the air on paper for several hours the silica powder is equally distributed between four 250 ml. beakers and dried at 300° to 320° C. for 20 hr. It is stored in a wide-mouth glass-stoppered bottle in a desiccator containing anhydrous magnesium perchlorate. The yield of dry silica is 80 to 82 gm. If equipment for drying the silica at 300° to 320° C. is not available it can be dried satisfactorily at 160° C. in four days.

Apparatus

The apparatus can be assembled from pieces readily available in most laboratories. That needed for separation of the fermentation acids by partition chromatography is shown in Fig. 1. The burette holding the indicator solution is protected from the air by a soda-lime tube and is fitted with a side arm bearing a stopcock (not shown) for refilling. A 500 ml. aspirating bottle, also not shown in the figure, holds a reserve supply of indicator for filling the burette. It is also protected by a soda-lime tube and connected to the side arm of the burette by glass and Tygon tubing. Rubber tubing is avoided since it produces acid on standing in contact with dilute alkali, presumably by oxidation of the sulphur. An enlarged diagram shows how the main stopcock of the burette is grooved by filing to permit easy adjustment of the rate of flow of the indicator solution.

The apparatus used for titration consists of a 2 liter aspirating bottle containing 0.01 *N* sodium hydroxide connected by glass and Tygon tubing to a 10 ml. burette, with a stopcock-bearing side arm. Both the bottle and burette are protected from the air by soda-lime tubes. They are mounted on a large ring stand with a mechanical stirrer arranged so the glass stirrer-blade is 5 to 6 mm. from the bottom of an inverted 250 ml. beaker which is used as a platform for the 150 ml. beaker used in the titration. A bent glass tube with its outlet near the blade of the stirrer serves as an inlet for carbon-dioxide-free air. The tip of the burette should be well inside the 150 ml.

beaker when it is in position. The carbon-dioxide-free air is obtained by passing compressed air from the laboratory lines through a soda-lime tower in series with a gas-washing bottle filled with *N* sodium hydroxide. This stream

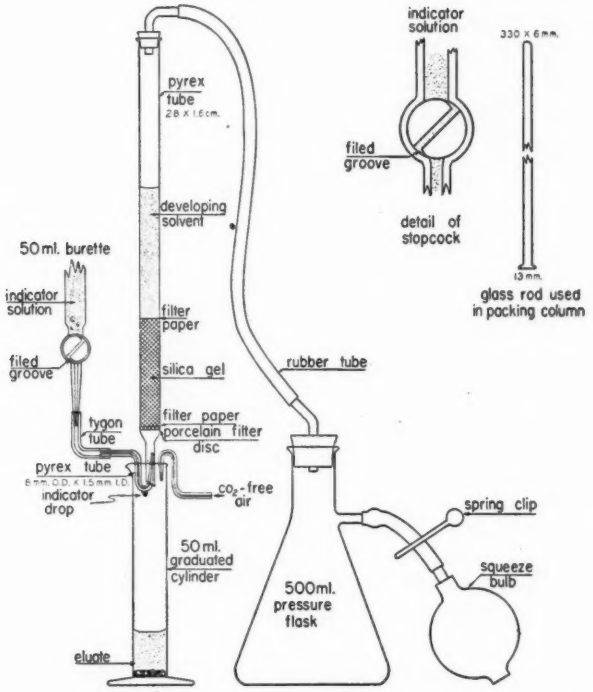

FIG. 1. *Apparatus used for chromatographic separation of the fermentation acids.*

of purified air is divided into three parts. One part is passed through a bubble counter at the rate of approximately 100 ml. per min. to provide the carbon-dioxide-free air for the apparatus shown in Fig. 1. Another part is used to protect the titrations while the main part escapes through a fritted glass gas dispersion tube immersed in a 1 liter Erlenmeyer flask containing distilled water. This serves as a safety valve and provides a supply of carbon-dioxide-free water for use in the titrations or for preparing the reagents. Water aerated for 10 to 20 min. in this apparatus is practically free of carbon dioxide.

Procedure

(1) *Preparation of the Acid Fraction for Partition Chromatography*

The fermentation solution is cleared with zinc hydroxide and 100 ml. extracted with ether to remove 2,3-butanediol and other neutral compounds as previously described (10). This is done in order to obtain a fraction for the

determination of 2,3-butanediol rather than to remove any substances that might interfere in the determination of the acids. The aqueous solution left from this extraction is then acidified with an excess of 5 *N* hydrochloric acid (5 to 10 ml.) and extracted for a further 24 hr. as before using a fresh extraction flask containing 50 ml. of anhydrous, reagent grade, ethyl ether but omitting the 20 ml. of water (10). The extract is thoroughly mixed with 25 ml. of *tertiary* amyl alcohol and transferred to a 100 ml. glass stoppered volumetric flask. The mixture is usually monophasic at this stage and ethyl ether is used to rinse out the extraction flask and make the extract to volume. If droplets of water are still apparent a further 10 ml. of *tertiary* amyl alcohol is used. If the fermentation solution is suspected to contain less than 2 milli-equivalents of acids per 100 ml. the extraction is performed with only 25 ml. of ether in the extraction flask and the extract made to 50 ml. using 12.5 ml. of *tertiary* amyl alcohol. The extract is stored in a refrigerator until it is convenient to perform the analysis. It is stable for at least two weeks.

In testing the method given in this paper synthetic mixtures of the acids were used. Stock solutions were prepared in the desired solvent (usually ether containing 25% of *tertiary* amyl alcohol) from samples of acids of at least 98% purity. The strength of these solutions was determined by titration and then mixtures were prepared from them volumetrically in order to obtain acid fractions of known composition. The best grade of 85% lactic acid available commercially is not chromatographically homogeneous; it gives a band amounting to approximately 10% of the total acidity which leaves the column before the main lactic acid band. Consequently the lactic acid stock solutions were made by dissolving recrystallized lithium lactate in water, acidifying with hydrochloric acid and extracting with ether as outlined above.

(2) *Preparation of Column for Partition Chromatography*

The chromatogram tube used, shown in Fig. 1, is prepared with the perforated porcelain plate and filter paper disk in position at the bottom. The disks are cut from a fast filter paper with a sharp cork borer. They are slightly larger than the internal diameter of the tube. Three grams of silica, prepared as described above, is weighed to the nearest 0.1 gm. and then mixed with 4.5 ml. of 0.01 *N* hydrochloric acid in a 100 ml. beaker. The silica is stirred with a stainless steel spatula as the acid is added drop by drop. After addition of the acid is complete the mixture is thoroughly blended by mixing and vigorously rubbing it against the sides of the beaker with the spatula for three to four minutes. When this is completed the silica should be a powder with a tendency to adhere to the wall of the beaker. It is slurried with approximately 20 ml. of BeB_5 but quantitative transfer is not necessary. The flocculent suspension in the upper half of the tube is stirred with a glass rod to release any trapped air bubbles. A disk of filter paper is now forced down on top of the gel by the flat-headed glass rod shown in Fig. 1. This rod should have a head only slightly less in diameter than the inside of the chromatogram tube. A firm pressure is maintained until the silica has been

compressed to a length of 8 cm. The supernatant liquid is agitated by raising and lowering the rod and quickly decanted to remove any particles that may have been left in the upper part of the tube. The column is now ready for separation of the acids. If it is inconvenient to start an analysis at once some fresh BeB_5 is poured into the tube; care must be taken at all times to prevent the column from drying out and admitting air. This method of packing gives a firm column that is not easily disturbed by pouring liquids in in or out of the chromatogram tube and that shows little change of length during an analysis although pressures up to 50 cm. of mercury are applied. These columns are not subject to cracking.

(3) *Chromatographic Separation and Estimation of the Fermentation Acids*

Before going into detail it is perhaps advisable to give a short description of the procedure and the principles involved. The total acidity of an aliquot of the acid mixture is determined by titration of a small aliquot, and then another aliquot of suitable size is pipetted into a column of silica gel, prepared as described above. As the acid fraction is forced into the column the water held by the silica gel extracts the acids from the organic solvent, those with partition coefficients most in favor of the aqueous phase being extracted most readily. When fresh solvent is forced through the column the acids are extracted back into the solvent phase and pass down the column moving back and forth between the organic and aqueous phases. The acids with partition coefficients most in favor of the organic phase will move most rapidly. As a result the acids separate into bands in the column and the eluate* is made up of fractions containing a single acid separated by fractions free of any appreciable amount of acid. A detailed mathematical description of this separation technique is given by the originators (9). The acids will come out of the column in the order butyric, acetic, formic, succinic, and lactic. The different acid fractions are collected separately and the amount of acid in each is quantitatively determined by titration with sodium hydroxide. In order to know when to change the receiver a slow stream of faintly alkaline phenol red solution is continuously fed into the eluate. The acids are identified by the amount of solvent required to bring them through the column, which is a measure of the partition coefficient. The detailed procedure is as follows.

The total acidity of the acid fraction prepared as described above (usually a mixture of butyric, acetic, formic, succinic, and lactic acids) is determined by titration of a 1 to 2 ml. aliquot to the phenol red end point with 0.01 *N* sodium hydroxide. This titration is performed with 80 to 100 ml. of water containing three drops of the 0.05% phenol red in the 150 ml. beaker of the titration apparatus described above. The tip of the burette should be below the surface and titration finished by adding 0.02 ml. portions of alkali until

* *The word "eluate" is used to describe the organic solvent that has passed through the column.*

a permanent red-pink color is obtained. In this and all other titrations a stream of carbon-dioxide-free air is continuously bubbled through the mechanically stirred solution.

The supernatant solvent (BeB_5) present in the chromatogram tube is poured out and an accurately measured aliquot of the acid fraction not exceeding 2 ml. (preferably 1 ml. or less) and containing no more acid than can be neutralized by 10 ml. of 0.01 *N* alkali is pipetted onto the silica gel. When doing this the tip of the pipette is held close to the gel and the liquid distributed equally over the surface. Gentle air pressure is applied to the chromatogram tube until the top of the column just barely becomes free of mobile liquid. Care must be exercised at this point to prevent air from getting into the gel. The moment the filter paper on the top of the column breaks through the surface of the liquid the pressure is quickly released by removing the rubber stopper at the top of the chromatogram tube. This is done with the left hand by pushing the stopper sharply to one side with the thumb and index finger while clasping the column with the other fingers. As soon as this operation is complete a 1 ml. portion of the solvent (BeB_5) is added from a pipette, in such a way as to wash down the inside wall of the tube. The receiver is changed to an empty one at this point and the solvent then gently forced into the gel and followed with another 1 ml. portion in the same way. The chromatogram tube is then nearly filled with the developing solvent (BeB_5) and pressure applied until the liquid is flowing through the column at the rate of 3 to 4 ml. per min. (*ca.* three drops per sec.). The indicator solution (0.0025% phenol red in 0.001 *N* sodium hydroxide) is made to flow at approximately one-tenth this rate. The amount of indicator added to each fraction is determined by reading the burette to the nearest 0.1 ml. each time the receiver is changed. Since 50 ml. graduates are used to receive the eluate the amount of developing solvent in each fraction is also known to the nearest milliliter and is recorded. This is necessary for making blank corrections as described below. The indicator drop (see Fig. 1) is watched in order to see when the eluate contains appreciable amounts of acid. If butyric acid is present this drop should turn yellow when a total of approximately 6 ml. of liquid has collected in the receiver. After 5 to 7 ml. more has collected it usually turns pink again; this indicates that the butyric acid is out. A total of 22 ml. is collected and titrated whether the indicator drop has shown acid or not, since as much as 0.30 ml. of 0.01 *N* acid may escape detection. The receiver is changed, the burette read, and development with BeB_5 continued, the chromatogram tube being filled with fresh solvent as required to prevent the column from going dry. After 10 to 20 ml. has been collected the acetic acid is removed in the next 18 to 20 ml. of eluate. A total of 40 to 43 ml. is collected (at least 3 ml. after indicator becomes pink again). The variability in the time of appearance of acetic acid is caused by variation in the amount of acid mixture put on the column. Since the ether – amyl alcohol mixture is a fairly strong developing solvent, analysis of a 2 ml. aliquot results in the acetic acid being carried farther down the column

initially than if a smaller aliquot of the acid fraction is used. When the receiver is changed, after collection of the acetic acid fraction, the supernatant liquid left in the chromatogram tube is *all poured out* and replaced by BeB_{10}. Pressure is again applied and the formic acid fraction (32 ml.) collected. The formic acid starts to come out after 12 ml. and is usually removed by 18 ml. more of eluate. The receiver is changed and the solvent decanted and replaced by BeB_{25}. After approximately 12 ml. has been forced through the column the succinic acid is removed in the next 8 ml. of eluate, a total of 23 ml. being collected. The receiver is changed again and the lactic acid collected in the next 30 ml. It usually starts to come out at 5 ml. and is out by 24 ml. This completes the separation of the acids. The BeB_{25} is poured out and replaced by BeB_5; after 15 ml. of this has passed through the column it is ready for the next analysis. Each acid fraction is titrated separately after transferring to the 150 ml. beaker of the titration apparatus using three 20 ml. portions of carbon-dioxide-free water. The 0.01 *N* alkali is run in at a fairly fast rate until the indicator turns pink (in the aqueous phase). The acid in the benzene phase is gradually extracted and the indicator changes back to yellow. More alkali is added intermittently in gradually decreasing amounts, a knowledge of the exact amounts being gained by experience in observing the rate of change of color. The titration is finished by adding 0.02 ml. portions until a pink-red end point is obtained which persists for more than one minute with thorough mixing of the two phases. There is a tendency to under-titrate these fractions until experience is gained with the method. If larger volumes of solvent are to be titrated it is important to make sure enough water is added to give a suspension of the oil-in-water type, rather than *vice versa*, otherwise no end point will be detected, until it is badly overshot.

In practice a fraction is titrated while the next one is being collected, two receivers being used interchangeably. The titrations must be corrected for the alkali in the indicator solution (0.001 *N*) and the "blank" acidity of the solvent. The latter is determined by titrating the developing solvents after they have been equilibrated with the 0.01 *N* hydrochloric acid or preferably after forcing them through a column prepared as used in the analysis. Usually these corrections almost cancel each other but they must be worked out to the nearest 0.01 ml. The alkali is normally present in slight excess and traces of acids in a fraction are determined by back-titration with 0.01 *N* hydrochloric acid. Since the total number of milliequivalents of acid put on the column is known, it is a simple matter to calculate the per cent recovery. If low, this means that some acid more difficult to extract from water than lactic acid was present in the mixture and the development should be continued using BeB_{50} or some other strong solvent mixture. When working with small amounts of acids it should be remembered that a small amount of hydrochloric acid is extracted when acid fractions are prepared by continuous ether extraction. This does not interfere in the determination of the individual acids but makes the "total acids" figure high and may lead one to think some

other organic acid is present. The amount of hydrochloric acid in 1 ml. of the acid fraction is roughly equivalent to 0.03 ml. of 0.01 *N*. This is left on the column during an analysis.

The total time required to develop the partition chromatogram described here is about 45 min. It is customary to analyze three acid fractions completely in about three hours.

The data given above for volumes of developing solvent required to remove the acids from the column are given as a guide but anyone applying this method to unknown solutions should first analyze a synthetic mixture to be more sure of his results. With a constant volume of the acid fraction added to the gel the volumes of solvent required can be reproduced within 1 ml. Actually it would be possible to analyze samples without use of the indicator stream but in fermentation solutions there is always danger of unexpected acids being present.

A column is used for three to four analyses and then discarded. Theoretically it could be used indefinitely but since the acid mixture is not fully saturated with water some of the aqueous phase is removed from the top of the column each time a new sample is analyzed. A column can be preserved under BeB_5 for several days by plugging the exit tube to prevent flow of solvent and then corking the top. Flow of solvent can be prevented by merely stoppering the top but this is not advisable since it allows the bottom of the column to dry out.

(4) *Application of the Method to Mixtures Containing Propionic Acid*

The procedure described above was worked out for a mixture of butyric, acetic, formic, succinic, and lactic acids, which are the only acids encountered so far in 2,3-butanediol fermentations. Since propionic acid is formed in good yield in some other bacterial fermentations a study has been made of the effect of this acid on the determination of those mentioned. When the regular extraction and development procedure is used this acid is found in the butyric acid fraction owing to the strong developing action of the solvents used. If the butyric acid band gives more than 7 ml. of eluate the presence of propionic acid should be suspected. In order to get a reliable estimate of the amounts of each of these acids it is necessary to obtain them in solution in chloroform containing 5% of *n*-butanol (CB_5). Transference of these acids to this solvent should be easily effected by neutralizing a 50 ml. aliquot of the acid fraction with sodium hydroxide, evaporating to dryness on a steam bath, and extracting the residue with CB_5 after mixing it with potassium acid sulphate, as described by Elsden (2). The other acids will be partially extracted but will not interfere in the analysis. Columns prepared as described above will resolve synthetic mixtures of butyric, propionic, acetic and formic acids, in CB_5, if operated as follows.

The sample is washed in and development started with chloroform. After 7 ml. of eluate has been collected the butyric acid is obtained in 8 ml.; after a further 8 ml. of nonacidic eluate the propionic is removed by 20 ml. The

solvent is then changed to BeB_5; after 25 ml. of neutral eluate the acetic acid is removed in 18 ml. The formic acid is then removed by BeB_{10} in a 16 ml. fraction obtained after 14 ml. of acid-free eluate. The acid fractions are titrated, as usual, to obtain a quantitative estimation of each acid. In practice, formic acid would not be determined in this fraction; the column would be regenerated by BeB_{25} after removal of the acetic acid.

Results and Discussion

The procedure described in this paper differs from previous applications of partition chromatography in some details. Changes have been made in the method of preparing the silica, the technique of packing and operating the column, and in the indicator solution, the developing solvents, the solution used to wet the silica, and the apparatus. Some of the changes made are necessary if resolution of the particular acid mixture obtained in fermentation solutions is to be obtained while other changes, although not absolutely essential, improve its speed or accuracy. The remainder of this paper is devoted to a discussion of results obtained by the method finally adopted and the reasons for modifying the technique of previous workers.

The successful separation of acids by partition chromatography depends primarily on having silica with suitable properties that can be reproduced from batch to batch. The original method of Gordon, Martin and Synge (3) has been modified several times (5, 6, 14) but all workers have used commercial water glass, which varies considerably from one batch to another (4). In an attempt to develop a method that will give reproducible batches of silica the writer has also modified the original procedure (3) by substitution of a purified grade of sodium metasilicate for water glass, by control of the precipitation temperature within specified limits, and by adoption of a higher temperature for drying the silica. The details of the procedure evolved are given in the Experimental Section. Twelve different batches have been prepared from different bottles of sodium metasilicate including the product of two different manufacturers (Merck's and the General Chemical Co.) and all have been found suitable. The need for control of the precipitation temperature is demonstrated by the results in Table I. The water absorbing capacity and apparent fineness of the silica are highest at the lowest precipitation temperatures. This is an effect opposite to that observed by Tristam (14), who found that silica prepared from water glass gave the most porous gels when precipitated at low temperatures. The precipitation temperature adopted in the present work (36° to 38° C.) is a compromise between that giving gels with a reasonably good filtration rate and that giving gels so coarse that the acids are not efficiently separated. It is necessary to cool the solution during the precipitation, otherwise the heat of neutralization will raise the temperature high enough to give gels that are too coarse for a good separation of lactic and succinic acids.

TABLE I

EFFECT OF PRECIPITATION TEMPERATURE ON PROPERTIES OF SILICA GEL

Temperature during precipitation of the silica, ° C.	Ml. of 0.01 *N* hydrochloric acid used to wet silica (3 gm.)	Length of column, cm.	Rate of flow of solvent, ml./min.	
			Column under pressure of 20 cm. of BeB_5	Column under 20 cm. of BeB_5 + 50 cm. of Hg.
28-32	4.0 5.0	9.2 9.2	0.08 0.24	2.0 4.5
36-38	4.0 5.0	8.4 8.4	0.16 0.36	3.1 5.6
56-74	4.0 5.0	6.1 *	0.34 —	7.2 —

* *This sample will not absorb 5 ml.; too sticky to use.*

The silica obtained has a higher capacity for binding water than that made by previous workers. Good separations of the fermentation acids have been obtained using it wetted with 166% of its weight of water (as 0.01 *N* hydrochloric acid). Other workers used gels wet with only 53% of water (3, 4, 9, 14) while the highest amount used previously is 100% (6). The gels used in the present work are regularly wetted with 150% and give too slow a filtration rate when wetted with smaller amounts. It is necessary to pack the columns by compression with a piston, rather than depending on gravitational forces as previous workers have done. Air pressure must be applied during development to get a convenient rate of filtration. The use of pressure is really convenient since it gives some control over the rate of flow of solvent and permits rates four times as rapid as those used by previous workers to be employed although the columns are intrinsically slower. It is felt that use of the method of packing and developing the chromatogram given above is important since otherwise the most efficient gels prepared would have been discarded as being too slow or for giving weak columns liable to crack.

Most workers have detected the position of the acid bands in the column by incorporating indicators such as methyl orange, bromocresol green, or alphamine-red R (7) into the water used to wet the silica. This is unsatisfactory since the indicators leach more readily than many organic acids, since some indicators are not sensitive enough to indicate the weaker acids (i.e., butyric), and since it is desirable to wet the silica with a dilute mineral acid in order to prevent tailing of the bands (6, 8). Isherwood introduced the use of thymol blue used externally to overcome these objections (6). He wet the silica with 0.5 *N* sulphuric acid to suppress tailing. In the present work it was found necessary to change this to 0.01 *N* hydrochloric acid to prevent decomposition of the formic acid (see Table II). Phenol red has been substituted for thymol blue because it gives a more accurate end point for the titration. The apparatus has been modified to permit accurate measurement

TABLE II

STABILITY OF FORMIC ACID IN SILICA GELS

Solution used to wet silica*	Method of preparation of silica**	Time in gel, hr.	As ml. of 0.01 *N*		% Recovered
			Formic acid added	Formic acid recovered	
0.5 *N* H_2SO_4	Isherwood's	1.7	4.41	3.82	86.6
"	"	3.7	4.41	2.68	60.6
"	"	17.0	4.41	0.88	19.9
"	Modified procedure	3.0	10.98	8.80	80.1
"	"	19.0	10.98	3.74	34.0
0.01 *N* HCl	"	3.0	10.98	11.00	100.2
"	"	19.0	10.98	10.11	92.2
Water	Isherwood's	2.4	4.41	4.50	102.1

** In wetting silica prepared by Isherwood's method (6), 3 ml. of solution was used, while 4 ml. was used for silica prepared by the modified procedure.*

*** The "modified procedure" is that given in detail in this paper.*

of the indicator added to each fraction, to minimize holdup in the detection system and to protect the alkaline indicator solution from the carbon dioxide of the ambient atmosphere.

Mixtures of the fermentation acids of known composition can be analyzed with ± 2% error, as shown in Table III. The chief difficulty lies in titration of the small amounts of acid involved. Particular attention has been paid

TABLE III

DETERMINATION OF FERMENTATION ACIDS IN SYNTHETIC MIXTURES BY PARTITION CHROMATOGRAPHY*

Expt. No.	As ml. of 0.01 *N*											
	Added						Recovered					
	Butyric	Acetic	Formic	Succinic	Lactic	Malic	Butyric	Acetic	Formic	Succinic	Lactic	Malic
1	—	—	—	3.34	3.46	—	—	—	—	3.28	3.50	—
2	—	—	—	1.33	10.39	—	—	—	—	1.32	10.37	—
3	—	—	—	10.02	1.73	—	—	—	—	10.01	1.73	—
4	—	—	—	16.70	8.65	—	—	—	—	16.72	8.61	—
5	3.74	4.41	4.38	2.67	3.46	—	3.69	4.40	4.30	2.65	3.48	—
6	1.49	4.42	1.74	1.29	0.69	—	1.51	4.44	1.72	1.29	0.69	—
7	0.75	0.89	8.76	1.29	1.38	—	0.76	0.93	8.74	1.29	1.36	—
8	1.49	1.76	1.75	2.58	6.92	—	1.51	1.73	1.74	9.50		—
9	0.74	0.88	0.87	1.29	3.46	—	0.74	0.89	0.87	1.28	3.44	—
10	1.86	2.25	2.34	1.78	1.81	—	1.87	2.25	2.34	1.77	1.80	—
11	1.55	1.87	1.95	1.45	1.54	2.50	1.53	1.82	1.94	1.47	1.53	2.46

** Acids were dissolved in ethyl ether containing 25% of tertiary amyl alcohol.*

to the determination of lactic and succinic acids, which, of all pairs of fermentation acids studied, are the most difficult to separate. Attempts to separate them using chloroform–butanol mixtures have failed (1) but they can be

separated fairly well if mixtures of butanol with aromatic hydrocarbons (benzene, toluene, xylene) or methyl isobutyl ketone are used. In the first four experiments (Table III) they were put on the column in 1 ml. of solvent and the column was developed with BeB_{25} only. Under these conditions a sample containing twice as much acid as recommended in the procedure could be analyzed successfully. However when the full development procedure is used a good separation between these acids is not obtained if the column is overloaded (Expt. 8, Table III). In Expt. 9, the same mixture used as in Expt. 8 was analyzed successfully by using one-half the quantity. That is why it is recommended that the sample taken for analysis contain not more than 0.1 milliequivalents of acid.

As mentioned above, the purpose of adding a strong acid to the aqueous phase is to suppress ionization of the fermentation acids and thus prevent tailing. If this condition is realized the concentration of acids in the eluate should give a curve resembling the normal curve of error when plotted against volume of eluate (9). Such a concentration curve is shown in Fig. 2, for the

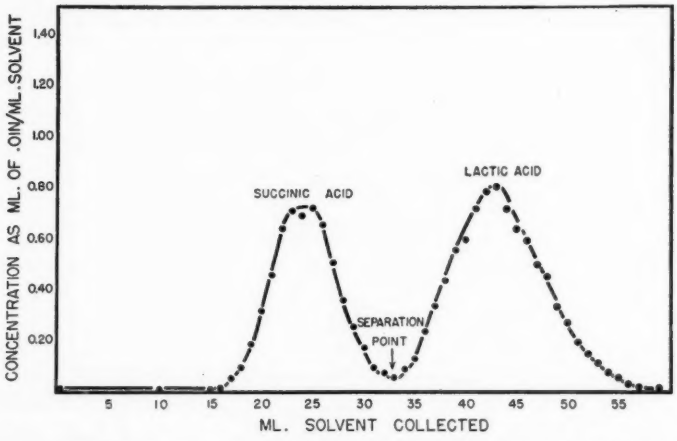

FIG. 2. *Concentration curves for chromatographic separation of succinic and lactic acids.*

separation of succinic and lactic acids. It is apparent that there is little sign of tailing even with these comparatively strong acids, and consequently that the gel is acting according to the theory of liquid–liquid extraction (9). In obtaining the data for this figure the column was overloaded 46% above the recommended amount in order to have more accurate titrations for the numerous small fractions. The amount of succinic acid added was equivalent to 5.93 ml. of 0.01 *N* (recovered 5.92) while the lactic acid added was equivalent to 8.66 ml. (recovered 8.65). It is apparent from Fig. 2 that the separation is not quite complete in spite of the good results obtained. The method of indication used in this paper is deliberately made insensitive

enough to detect separations like this, and thus give answers accurate within ± 2%, where a highly sensitive method of detection would show only one acid band.

The procedure given in the experimental section for analysis of mixtures containing propionic acid is based on the results shown in Table IV. When the regular extraction and development procedure was used propionic acid

TABLE IV

DETERMINATION OF VOLATILE ACIDS IN SYNTHETIC MIXTURES

Experiment No.	Solvent used in preparing mixture	Development procedure*	As ml. of 0.01 *N*							
			Added				Recovered			
			Butyric	Propionic	Acetic	Formic	Butyric	Propionic	Acetic	Formic
1	Ethyl ether plus	Regular procedure	2.07	0.68	2.48	2.92	2.76	Nil	2.48	2.91
2	25% *tertiary*		2.07	2.05	2.48	2.92	4.07	Nil	2.52	2.90
3	amyl alcohol		2.07	3.45	2.48	2.92	5.48	Nil	2.50	2.96
4	Ethyl ether plus	Carbon tetrachloride followed by regular procedure	2.07	0.68	2.48	2.92	2.10	0.65	2.47	2.90
5	25% *tertiary*		2.07	2.05	2.48	2.92	2.70	1.47	2.51	2.88
6	amyl alcohol		2.07	3.45	2.48	2.92	3.27	2.23	2.54	2.90
7	Chloroform plus	Carbon tetrachloride followed by regular procedure	2.04	0.74	2.33	2.55	0.71	2.08	2.31	2.55
8	5% *n*-butanol		3.40	2.24	—	—	1.95	3.71	—	—
9			3.23	2.04	—	—	2.01	3.26	—	—
10			5.42	—	—	—	3.62	1.80	—	—
11	Chloroform plus	Chloroform followed by regular procedure	5.42	—	—	—	5.41	—	—	—
12	5% *n*-butanol		3.23	2.04	—	—	3.24	1.98	—	—
13			2.04	0.74	2.40	2.55	2.06	0.73	2.39	2.51
14			2.04	3.67	2.40	2.55	2.03	3.65	2.41	2.52
15			3.40	3.67	2.40	2.55	3.41	3.61	2.41	2.48

* *The "regular procedure" is that given in detail in the Experimental Section of this paper for mixtures not containing propionic acid; 1 ml. of acid fraction put on the column.*

was not separated from butyric acid (Expts. 1 to 3) but it had the effect of doubling the volume of acidic eluate collected as the butyric acid fraction. If the column is first developed with carbon tetrachloride, separate bands of butyric and propionic acid are formed (Expts. 4 to 6). The butyric band comes between 5 and 10 ml. of eluate; after changing to BeB_5 the propionic band is collected in 13 ml. after 9 ml. of neutral eluate. However, the figures for butyric acid are high while those for propionic acid are low by an equal amount. It appears that the acid band due to propionic has been distorted so that it gives two maxima. This can be explained as due to the strong developing action of the mixture of ether and amyl alcohol in which the acids were dissolved in these experiments. It carries them both far down the column but the propionic acid is partially extracted into the aqueous phase.

On developing with carbon tetrachloride part of the propionic acid is removed with the butyric while the most of the rest comes off quite a bit later. It should be possible to avoid this phenomenon by having the acids dissolved in a mixture with less developing action. Consequently the acids were dissolved in chloroform containing 5% of butanol, and the developing procedure, starting with carbon tetrachloride, was again applied (Expts. 7 to 10). The same type of behavior was found except this time it was the butyric acid that formed two bands. This is best shown in Expt. 10 in which butyric acid was the only one put on the column yet two distinct bands were formed. After 8 ml. of carbon tetrachloride was collected the indicator showed acid for 18 ml.; the solvent was changed to BeB_5 and after 10 ml. with "no acid" the eluate was acidic for 6 ml. more. It is inconvenient to use a weaker solvent for the acid mixture; however, one can expect to prevent this "band-splitting" by using a stronger developing solvent in this case. Therefore carbon tetrachloride was replaced by chloroform and in this way satisfactory analyses of mixtures of the volatile fatty acids obtained (Expts. 11 to 15).

All the results given above were obtained using synthetic mixtures of acids. However the procedure has been successfully applied to glucose solutions fermented by *Aerobacter aerogenes*, *Bacillus polymyxa*, *Bacillus subtilis* (Ford's type) and *Serratia marcescens* (work, as yet, unpublished).

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UNE NOUVELLE SYNTHÈSE DE L'ACIDE AMINOADIPIQUE[1]

PAR ROGER GAUDRY

Résumé

L'acide α-aminoadipique a été préparé avec un rendement de 48% à partir de l'acide α-amino-ε-hydroxycaproïque par oxydation de son dérivé benzoylé en acide α-benzoylaminoadipique, suivie d'hydrolyse du groupement benzoyle. Le dérivé phtalylé fut aussi préparé à partir de l'acide α-aminoadipique et de l'anhydride phtalique, de même que par oxydation de l'acide α-phtalimido-ε-hydroxycaproïque, mais cette dernière méthode ne donne que de faibles rendements.

Introduction

Récemment, Borsook et ses collaborateurs (2) ont démontré que l'acide α-aminoadipique est un produit de métabolisme de la lysine, et est même susceptible de remplacer celle-ci dans les milieux de culture de certains micro-organismes.

Mais l'acide α-aminoadipique n'est pas une substance facilement accessible. Il a d'abord été synthétisé par Sörensen (6), par condensation du phtalimidomalonate d'éthyle et du γ-chlorobutyronitrile, suivie d'hydrolyse, ensuite par Dieckmann (3), par réduction de l'acide α-oximinoadipique.

Nous avons pensé qu'il serait possible d'en élaborer une synthèse plus pratique à partir de l'acide α-amino-ε-hydroxycaproïque, dont nous avons récemment publié la synthèse (4), et qu'on peut maintenant préparer en grandes quantités à partir du dihydropyrane.

La méthode que nous avons utilisée est celle mise à point par Klostermann et Painter (5) pour la synthèse de l'acide aspartique à partir de l'acide α-amino-γ-hydroxybutyrique. Nous avons benzoylé l'acide α-amino-ε-hydroxycaproïque (I) avec le chlorure de benzoyle, et nous avons oxydé l'acide α-benzoylamino-ε-hydroxycaproïque (II) en acide α-benzoylaminoadipique (III) au moyen du permanganate de potassium en milieu alcalin. L'hydrolyse du groupement benzoyle par l'acide chlorhydrique dilué a donné l'acide α-aminoadipique (IV) avec un rendement de 48% à partir de l'acide α-amino-ε-hydroxycaproïque (I).

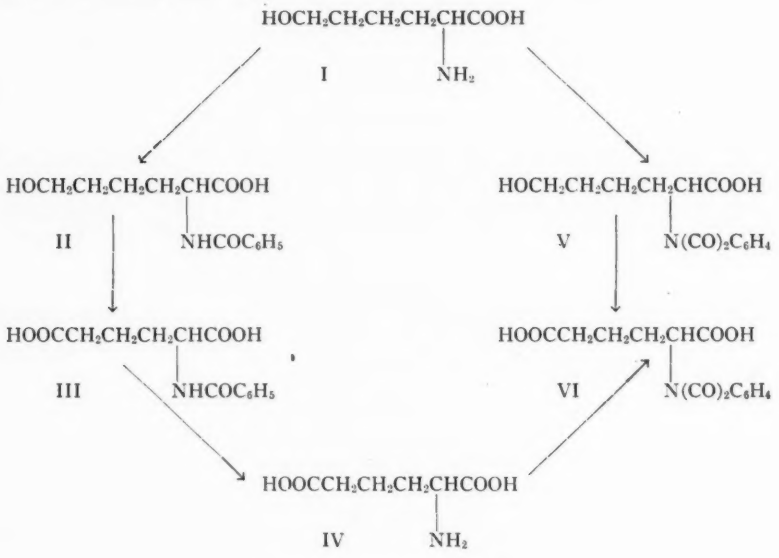
Une autre possibilité consistait à utiliser le dérivé phtalylé (V) de l'acide α-amino-ε-hydroxycaproïque, au lieu du dérivé benzoylé. Nous l'avons préparé en chauffant un mélange d'anhydride phtalique et d'acide aminé, d'après la méthode de Billman et Harting (1), et nous l'avons oxydé en acide α-phtalimidoadipique (VI), identique au produit obtenu en chauffant l'acide α-aminoadipique avec l'anhydride phtalique. Mais parce que l'acide α-phtalimido-ε-hydroxycaproïque ne s'obtient qu'avec un rendement de 8%, à com-

[1]*Manuscrit reçu le 7 octobre 1948.*

Contribution du Département de Biochimie de la Faculté de Médecine, Université Laval, Québec, Qué.

parer avec 80% pour le dérivé benzoylé, cette modification de la méthode ne présente qu'un intérêt théorique.

Comme c'est par hydrolyse de la 5-δ-hydroxybutylhydantoïne que nous obtenons l'acide α-amino-ε-hydroxycaproïque (4), il eut été avantageux d'oxyder directement cette hydantoïne en l'hydantoïne correspondant à l'acide α-aminoadipique, sans passer par le dérivé benzoylé. Malheureusement, nos essais furent infructueux.



Partie expérimentale

Acide α-benzoylamino-ε-hydroxycaproïque (II)

Dans un ballon à trois cols de 500 ml. plongé dans un bain de glace et d'eau, muni d'un thermomètre, d'un agitateur et de deux entonnoirs à décanter, on place 14.7 g. (0.1 mole) d'acide α-amino-ε-hydroxycaproïque, 100 ml. d'une solution normale de soude caustique et quelques gouttes d'une solution de phénolphtaléine. En maintenant le mélange entre 0 et 10° C., on ajoute goutte à goutte, simultanément, 12 ml. de chlorure de benzoyle et 100 ml. de soude normale, en réglant les vitesses d'addition de telle sorte que le mélange réactionnel soit constamment alcalin. On continue d'agiter pendant une heure, on acidifie avec 30 ml. d'acide chlorhydrique concentré, on porte à la glacière jusqu'à ce que le précipité blanc soit cristallin, on le filtre à succion, on le lave dans un mortier avec de l'éther, on le filtre à nouveau, et on le sèche à l'air. Rendement brut, 24.5 g., 97.7%. Pour le recrystalliser, on le dissout dans un petit volume d'alcool éthylique bouillant (environ 25 ml.), on refroidit et on ajoute un grand volume d'éther (250 ml.). Quand la crystallisation est terminée, on filtre à succion le précipité, on le lave à l'éther sur le filtre et on

le sèche. Rendement: 21 g., 80%. P.f. 144° à 146°.* Calculé pour $C_{13}H_{17}O_4N$: N, 5.57%. Trouvé: N, 5.60%.

Acide α-benzoylaminoadipique (*III*)

A une solution de 25.1 g. d'acide α-benzoylamino-ε-hydroxycaproïque (0.1 mole) dans 200 ml. d'une solution normale de soude caustique, on ajoute lentement 21 g. de permanganate de potassium, en agitant constamment, et en ayant soin de tenir le mélange réactionnel dans un bain d'eau froide (environ 10° C.). Quand la coloration due au permanganate est entièrement disparue (ce qui nécessite trois à quatre heures), on filtre à succion le bioxyde de manganèse, on acidifie la solution incolore avec 50 ml. d'acide chlorhydrique concentré, on mélange et on porte aussitôt à la glacière. Lorsque la crystallisation est terminée, on filtre à succion, et on recrystallise aussitôt en dissolvant le précipité dans 250 ml. d'eau bouillante. Après refroidissement, on filtre et on sèche à l'air. Rendement: 20 g., 75%. P.f. 184° à 185°. Litt. (6): 184°. Calculé pour $C_{13}H_{15}O_5N$: N, 5.28%. Trouvé: N, 5.32%.

Acide α-aminoadipique (*IV*)

A une solution de 26.5 g. d'acide α-benzoylaminoadipique (0.1 mole) dans 300 ml. d'eau bouillante, on ajoute 200 ml. d'acide chlorhydrique concentré, et on fait bouillir à reflux pendant six heures. Après refroidissement, on filtre à succion l'acide benzoïque, et on évapore le filtrat dans le vide. On dissout le résidu huileux dans 200 ml. d'alcool éthylique absolu bouillant, on ajoute 12 ml. de pyridine, et on porte à la glacière. On filtre à succion le précipité blanc, et on le lave au mortier avec de l'alcool absolu pour éliminer les dernières traces de pyridine, et on le sèche à l'air. Rendement: 12.9 g., 80%. P.f. 185° à 189° avec dégagement de gaz. Calculé pour $C_6H_{11}O_4N$: N, 8.69%. Trouvé: N, 8.47%.

Acide α-phtalimidoadipique (*VI*) *à partir de l'acide α-aminoadipique*

On mélange intimement dans un mortier 1.6 g. d'acide α-aminoadipique et 1.4 g. d'anhydride phtalique et on chauffe à 180° C. pendant 10 min. dans un bain d'huile. Par refroidissement on obtient un solide blanc qu'on recrystallise de l'eau bouillante. Rendement: 2.1 g., 70%. P.f. 227° à 229°. Calculé pour $C_{14}H_{13}O_6N$: N, 4.81. Trouvé: N, 4.79%.

Acide α-phtalimido-ε-hydroxycaproïque (*V*)

On chauffe à 180° C. dans un bain d'huile, pendant 10 min., un mélange intime de 10 g. d'acide α-amino-ε-hydroxycaproïque et de 10 g. d'anhydride phtalique. On dissout la masse refroidie de l'alcool dilué chaud. Par refroidissement, il se sépare une abondante couche huileuse. On décante alors la solution surnageante, qu'on laisse s'évaporer dans une capsule de porcelaine. On obtient alors un fin précipité blanc, crystallin. Rendement: 1.3 g., 8%. P.f. 162° à 163°. Calculé pour $C_{14}H_{15}O_5N$: N, 5.05%. Trouvé: N, 5.04%.

**Les points de fusion ne sont pas corrigés.*

Acide α-phtalimidoadipique (VI) à partir de l'acide α-phtalimido-ε-hydroxycaproïque

A une solution de 3 g. d'acide α-phtalimido-ε-hydroxycaproïque dans 50 ml. d'une solution demi-normale d'hydroxyde de sodium, on ajoute lentement 2.2 g. de permanganate de potassium, en maintenant le mélange à la température de la chambre. Après quatre heures d'agitation, on ajoute quelques gouttes d'alcool méthylique pour réduire l'excès de permanganate, on filtre le bioxyde de manganèse, on acidifie le filtrat limpide et incolore avec 5 ml. d'acide chlorhydrique concentré et on porte à la glacière. On filtre à succion le précipité et on le recrystallise dans 25 ml. d'eau bouillante. Rendement: 0.5 g., 15%. P.f. 224° à 226°. Un point de fusion mélangé avec l'acide α-phtalimidoadipique obtenu à partir de l'acide α-aminoadipique a donné 226° à 227°.

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THE TESTING OF ACTIVATED CHARCOALS BY THE ISOPIESTIC METHOD[1]

By H. Sheffer,[2] K. T. Waldock[3] and J. B. Ferguson[4]

Abstract

The isopiestic method has been applied to the testing of activated charcoals. The ratios of vapor adsorbed at saturation were found to agree well with the ratios of the activities obtained by a flow method. The sorptive capacities of nine different activated charcoals for carbon tetrachloride, water, amyl chloride, benzene, methyl alcohol, *n*-hexane, mustard gas, and phosgene have been compared. A simple, rapid method for testing charcoals is suggested.

One of the standard methods for measuring the "activities" or sorptive powers of activated charcoals involves the use of a flow system (7, 3). Air containing a definite concentration of carbon tetrachloride is passed through a specified volume of charcoal until a detectable trace of vapor appears in the effluent air. From the weight of carbon tetrachloride adsorbed the "volume activity" (*V.A.* = grams of carbon tetrachloride adsorbed per 100 cc. of charcoal) is obtained. This empirical method has been criticized by Allmand and Manning (1) and by Chaney, Ray, and St. John (2), not only because of its tediousness, but because factors that bear no relation to the intrinsic character of the charcoal may affect the results considerably.

In 1941 Dr. R. A. Robinson suggested that an attempt be made to apply the isopiestic equipment of these laboratories to the study of activated charcoals. Sinclair (10) and Robinson and Sinclair (8) made the greatest improvements in the isopiestic or isotonic method in their application of it to the measurement of vapor pressures of aqueous solutions. The historical and theoretical development of this method has been summarized by Janis and Ferguson (5). The underlying principle is the equilibration of solutions to the same vapor pressure by distillation of vapor from one solution to another under vacuum. It was thought that vapor distribution, in this manner, among charcoals would yield information as to their relative sorptive capacities.

The present investigation deals with such equilibrium measurements and their significance. A simple method is suggested for testing activated charcoals rapidly and easily, the results having at least as much value as the standard *V.A.* measurement.

Experimental and Results

The Isopiestic Method

Since the accuracy of the vapor pressure measurements for aqueous solutions was not required for the charcoals, the copper block and silver dishes used by

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Sheffer, Janis, and Ferguson (9) were not employed in these experiments. The charcoal samples were weighed into weighing bottles which were placed in a desiccator along with the required amount of carbon tetrachloride. The desiccator was carefully evacuated and allowed to stand until equilibrium was attained, after which dry air was allowed into the desiccator and the gain in weight of the charcoal samples obtained. It was found that a grease made by partially saponifying linseed oil was satisfactory for the retention of carbon tetrachloride vapor. When it was desired to place the desiccator in water in a thermostat, this greasy "soap" was used on the inner half of the flange of the desiccator while ordinary stopcock grease was used on the outer half. In most of the experiments it was found that thermostatically controlling the desiccator was unnecessary. Particularly near saturation, slight variations in temperature affected all samples proportionately.

All charcoal samples used were air-dried at 110° C. to constant weight. The sample numbers and corresponding *V.A.*'s as determined by the supplier are given in Table I. The charcoals were all from coconut shell, activated in

TABLE I

COMPARISON OF ISOPIESTIC AND FLOW METHOD ACTIVITIES

Charcoal No.	Isopiestic ratio near saturation $\frac{(W.A.)_x}{(W.A.)_7}$	Average bulk density	Calculated *V.A.* (isopiestic)	Supplier's *V.A.* (flow method)
1	0.670	0.556	14.8	13.2
2	0.655	0.519	13.5	13.7
3	0.760	0.547	16.6	17.8
4	0.827	0.560	18.4	18.0
5	1.055	0.444	18.7	20.3
6	1.095	0.495	21.6	21.3
7	1.000	0.535	St'd.	21.3
8	1.085	0.502	21.7	21.5
9	1.100	0.529	23.2	23.6

different ways and sprayed with various catalysts. The carbon tetrachloride used was a C.P. grade dried over sodium hydroxide.

In a preliminary experiment one sample of charcoal was allowed to adsorb approximately 30% by weight of carbon tetrachloride vapor. This sample, together with an uncharged one of the same charcoal, were placed in a desiccator under vacuum. It was found that the carbon tetrachloride distilled from the charged to the uncharged sample until both were within 1% of the same concentration.

To obtain the rate of attainment of equilibrium, two samples of each of two different charcoals were placed in a desiccator, together with a dish containing the desired amount of carbon tetrachloride. The desiccator was then partially evacuated and allowed to stand until all the carbon tetrachloride was adsorbed by the charcoals (about 15 min.). The residual air was then removed by

further evacuation and the desiccator was placed in a thermostat until equilibrium was reached. The samples were periodically removed and weighed and when the concentration of like pairs agreed closely it was considered that equilibrium had been attained. In this way it was found that equilibrium was reached very slowly at low concentrations, e.g., at concentrations around 10% of the saturation value the samples were not at equilibrium even after four days. At concentrations in the neighborhood of 25% of saturation, two days were sufficient for attainment of equilibrium, this time decreasing to 24 hr. at 50% and to only two to three hours at saturation.

Charcoal No. 7 was arbitrarily chosen as a standard in the intercomparison of the various charcoal samples by the above method. The isopiestic ratio for two charcoals is defined as the ratio of the weights of adsorbed material per gram of charcoal at equilibrium, the weight for the standard being the divisor. In the isopiestic curves shown in Fig. 1 the isopiestic ratios are

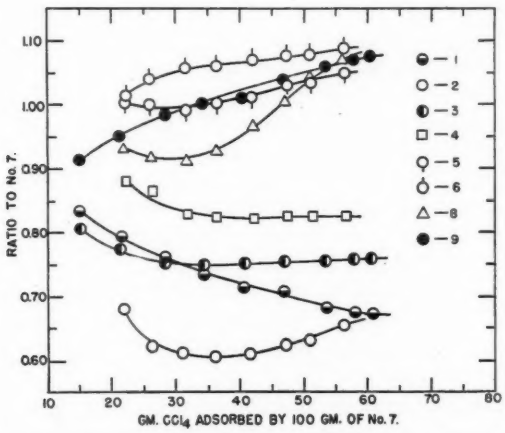

FIG. 1. *Isopiestic ratio curves with charcoal No. 7 as standard.*

plotted against the corresponding weight percentage (grams per 100 gm. of charcoal) for the standard, No. 7. It is evident from these curves that the charcoals vary considerably in their relative pickup over the range of partial pressures involved. The ratios corresponding to the lower partial pressures are of interest in considering the use of charcoals at low pressures. All the curves tend to flatten out near saturation and it is only in this region that ratios are obtained that are at all comparable with those obtained from the *V.A.*'s of the flow method.

In order to make this comparison it was necessary to obtain the bulk densities of the charcoals, since the isopiestic determinations were on a weight basis. The bulk density of each was determined by placing approximately 20 cc. of the charcoal in a 25 cc. graduate, tapping to maximum density, and

weighing the known volume. By this method the values were reproduced to better than 1%. The average values of the bulk densities (*B.D.*) are given in Table I.

The *V.A.*'s were calculated from the isopiestic results using No. 7 as a standard. If we define the weight activity, *W.A.* = grams carbon tetrachloride adsorbed per 100 gm. charcoal, near saturation, then the *V.A.*'s may be calculated using the following equation:

$$(V.A.)_x = \frac{(W.A.)_x}{(W.A.)_7} \cdot \frac{(B.D.)_x}{(B.D.)_7} \cdot (V.A.)_7 .$$

The values calculated in this manner are compared with those from the flow method in Table I. The discrepancy between the two methods amounts to about 11% for charcoal No. 1, but is much less for all the others.

The Flow Method

In order to determine the significance of the isopiestic results in relation to the *V.A.* values given by the charcoal supplier, a flow apparatus was set up. This apparatus was a simplified version of the type described by Mantell (7). The charcoal container was a glass tube 1 cm. in diameter. It was found that when the charcoal column was 15 cm. long, results were obtained that were comparable with those of the supplier's. The over-all rate of air – carbon tetrachloride flow was approximately 1000 cc. per min. in each measurement, the concentration of carbon tetrachloride being allowed to vary within limits as indicated in Column 2 of Table II. As before, the actual determinations were made on a weight basis and from these the *V.A.*'s were calculated. The results, given in Table II, indicate considerable variation in the *V.A.* values (Column 4) for each charcoal. Both the supplier's and the isopiestic *V.A.* values lie within this range of deviation.

It may be noted that no simple relation exists between the rate of adsorption and these variations. This point was further investigated using a sample of charcoal No. 3 at a rate of 0.0798 gm. per min. (approximately double the rate of adsorption), keeping the over-all rate of air the same as before. This gave a *V.A.* of 18.7 for No. 3, indicating that the radical change in rate did not materially alter the *V.A.* value.

It was found, however, that for a constant concentration of carbon tetrachloride, in the air stream, the *V.A.* increased as the air rate decreased, as might be expected. When the rate of adsorption was normal, halving the total flow was found to increase the *V.A.* of No. 3 to 20.9 and of No. 7 to 25.6. Although these values differ considerably from those obtained at the normal flow rate, their *ratio* is approximately the same as the ratio of the normal *V.A.*'s. The ratios, therefore, are less affected by the experimental conditions than the actual *V.A.*'s and it is the former that are given by the isopiestic comparison.

TABLE II

VARIATIONS IN ACTIVITIES FROM FLOW METHOD

Charcoal No.	Gm. CCl_4 adsorbed/min.	Weight activity	Volume activity	Average *V.A.*	Supplier's *V.A.*
1	0.0390	21.3	11.5		
	0.0410	22.3	12.4		
	0.0420	25.1	13.9	12.5	13.2
	0.0450	21.7	12.1		
3	0.0364	31.3	17.1		
	0.0370	33.8	18.5		
	0.0371	36.4	19.9	18.0	17.8
	0.0410	32.0	17.5		
	0.0458	30.8	16.9		
4	0.0398	32.0	17.9	18.4	18.0
	0.0450	33.8	19.0		
5	0.0335	46.5	20.7	20.9	20.3
	0.0340	47.5	21.1		
7	0.0355	43.8	23.4		
	0.0368	39.5	21.1	22.0	21.3
	0.0375	40.4	21.6		
8	0.0336	46.7	23.4		
	0.0375	48.5	24.3	22.9	21.5
	0.0375	43.7	21.9		
	0.0423	44.0	22.0		
9	0.0338	44.2	23.4		
	0.0350	47.1	24.9		
	0.0362	46.1	24.4	24.3	23.6
	0.0364	47.9	25.4		
	0.0376	45.0	23.8		
	0.0443	45.7	24.2		

In order to gain further information as to the significance of the *V.A.* values as obtained by the flow method, it was decided to obtain the concentration gradient of adsorbed carbon tetrachloride through the charcoal bed. For this determination the charcoal column was divided into four approximately equal sections by means of glass wool plugs, each section of charcoal being accurately weighed before and after testing. Although the true concentration gradient could not be obtained from so few measurements, the approximate shape of the curve was indicated. Typical results are shown in Fig. 2.

The general shapes of the curves are quite similar and it can be seen that the volume activities, which are, of course, proportional to the areas under each curve, are governed mainly by the top parts of the curves, i.e., where the charcoals are *nearly saturated*. This shows why the isopiestic ratios near saturation give comparable results. A comparison of the two curves for No. 3 in Fig. 2 shows that the charcoal in the first part of the tube is more nearly saturated at the higher adsorption rate, but that the gradients for the lower part of the tube are such that the areas and the *V.A.*'s are practically identical.

It has been pointed out from the results in Table I that the isopiestic *V.A.* for charcoal No. 1 was considerably higher than that from the flow method. In Fig. 2 it is seen from the gradient curve for No. 1, that there is a considerable

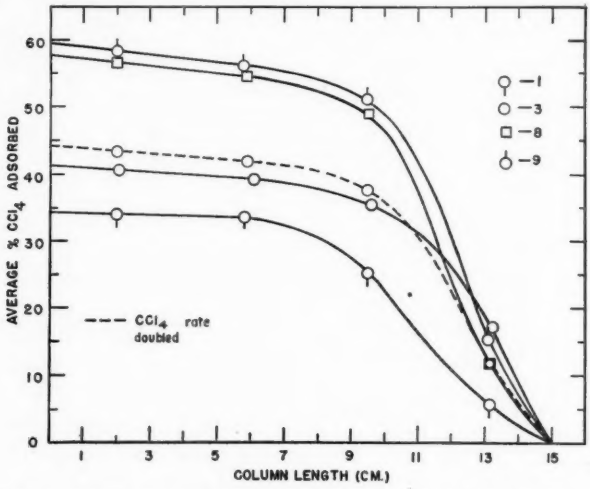

FIG. 2. *Concentration gradients of adsorbed carbon tetrachloride in charcoal columns after flow testing.*

area under the sloping part of the curve so that, in this case, the *V.A.* is not governed as much by the saturation value. If a longer charcoal column had been used, the relative increase in area under the upper part of the curve would have given results more comparable with the isopiestic values.

Procedure for Obtaining Isopiestic Ratios Near Saturation

Since the saturation values control the *V.A.*'s, it was desirable to determine the simplest procedure for obtaining these isopiestically. In Table III the

TABLE III

ISOPIESTIC ACTIVITIES NEAR SATURATION

Charcoal No.	Run 1		Run 2		Run 3		Run 4		Supplier's *V.A.*
	W.A.	*V.A.*	*W.A.*	*V.A.*	*W.A.*	*V.A.*	*W.A.*	*V.A.*	
1	46.5	14.2	39.9	14.4	45.5	15.3	47.1	15.6	13.2
2	48.0	14.0	38.2	13.7	44.6	13.9	46.9	14.5	13.7
3	54.3	16.7	47.5	17.0	53.4	17.9	55.2	18.1	17.8
4	56.8	17.8	49.8	18.2	51.3	17.7	53.0	17.8	18.0
5	76.0	18.9	65.9	19.1	71.2	19.6	74.9	20.0	20.3
6	76.8	St'd.	66.0	St'd.	74.0	22.4	77.2	22.9	21.3
7	73.5	22.0	62.3	21.7	70.2	23.0	71.5	22.8	21.3
8	75.0	21.1	64.6	21.2	68.0	21.0	70.4	21.2	21.5
9	72.5	21.6	63.0	21.8	72.5	St'd.	74.4	St'd.	23.6

weight activities obtained by various procedures are given. The *V.A.*'s, calculated from these by taking one charcoal as standard and using the bulk densities given in Table II, are also shown.

In *Run 1* approximately 3 gm. of each sample was used, two hours being allowed for the attainment of equilibrium at saturation. These samples were returned (without additional carbon tetrachloride) to the desiccator after weighing, and, after evacuation, allowed to stand a second two hours, after which the results given for *Run 2* were obtained. No. 6 was taken as standard.

In *Run 3*, 6 to 7 gm. of each charcoal was used and three hours were allowed for saturation, after which dried air was allowed into the desiccator and five minutes allowed for any condensed carbon tetrachloride to evaporate from the open weighing bottles. In *Run 4* these same samples remained in the evacuated desiccator overnight along with excess carbon tetrachloride. The results so obtained did not differ radically from those of Run 3 so that equilibrium must be closely approached in three hours.

To show that the discrepancies in the results are due to nonuniformity of the samples and not to lack of equilibrium, six samples of No. 5 were compared in the same manner as in Run 3. The weight activities at saturation after three hours were as follows: 74.8; 73.3; 72.7; 71.8; 71.7; 77.6. The excess carbon tetrachloride being removed, the samples were returned to the desiccator which was completely evacuated and allowed to stand in a thermostat for 48 hr. The following values were then obtained: 66.7; 65.1; 64.4; 64.2; 63.9; 68.8. The discrepancies were still of the same magnitude, indicating the nonuniformity of the average surface of the charcoal and the fact that equilibrium is rapidly approached.

Final Test Procedure

The procedure for Run 3 was adopted as most suitable because of its simplicity and reliability. A vacuum desiccator of 6½ in. internal diameter was used. In this vessel nine weighing bottles (40 by 50 mm.) fitted snugly in a circle. Approximately 7 gm. of charcoal was weighed into each dish. At the center of the desiccator was placed another dish containing an excess of carbon tetrachloride. The desiccator was evacuated slowly and carefully by means of a water pump and then allowed to stand for three hours. If kept in a draft-free location, it was found that thermostatic control was not required. If the temperature of the room varies much, it would be desirable to place the desiccator in a lagged box. After the three-hour period, dried air was slowly admitted into the desiccator through a capillary stopcock. It was noticed that some droplets of carbon tetrachloride condensed on the dishes during this operation. This was allowed to evaporate by exposing the open dishes to the air for five minutes before the lids were placed on the weighing bottles.

Weighing gave the ratios of the weight activities at saturation. With the additional determination of bulk density as described above, the ratios of the volume activities could be obtained.

It is advisable to use three samples of each charcoal and so obtain an average ratio for two charcoals against the standard. By using several desiccators many samples can be compared in the course of a day by this method.

The procedure suggested above has the greatest value for charcoals that have been activated in the same manner and have isotherms of similar shapes. Charcoals that have greatly differing isotherms will give different results between the flow method and the isopiestic method unless a very long charcoal column is used in the former (e.g., charcoal No. 1). In any case, the behavior of the charcoal at low partial pressures of the adsorbed vapor may be ascertained by obtaining isopiestic ratios at these low pressures in addition to the saturation values. If the charcoal is to be used under static conditions, the isopiestic saturation values would appear to be more meaningful than the standard flow method *V.A.*'s, which have such great dependence on the conditions of measurement.

Sorption of Other Vapors

The investigation was extended to include the determination of the isopiestic saturation value for water, amyl chloride, benzene, methyl alcohol, *n*-hexane, mustard gas, and phosgene in addition to the original carbon tetrachloride results. For the first five of these the same procedure was used to obtain equilibrium at saturation as for carbon tetrachloride, the time necessary varying from 2 to 48 hr., the actual time depending on the volatility of the liquid used.

In the case of mustard gas, which has a very low volatility, an attempt was made to expose more liquid surface by using a larger dish, but even then equilibrium was reached only after two weeks. With two samples of charcoal present instead of eight, equilibrium was attained in four to five days. Thus the charcoals adsorb the material very quickly, the lengthy time necessary being entirely due to the slow rate of evaporation of the liquid.

With phosgene, a gas at room temperature, it was necessary to modify the procedure somewhat. The phosgene tank was connected to a 2-liter bulb, then to a mercury manometer and the desiccator containing the charcoal samples. The system was completely evacuated and then phosgene was slowly admitted until the pressure was about 70 cm. More phosgene was allowed in as the mercury level changed until equilibrium was reached, indicated by no further movement of the mercury.

The experimental results are given in Table IV. Taking charcoal No. 7 as standard, the ratios of the isopiestic *V.A.*'s for each sample against that for No. 7 are given in Table V for the various vapors. The ratios for the different

TABLE IV

ISOPIESTIC SATURATION VALUES FOR VARIOUS VAPORS

Vapor	Gm. adsorbed/100 cc. for charcoal No.:				
	1	2	3	7	9
Carbon tetrachloride	26.2	24.4	30.2	38.2	39.4
Phosgene	24.3	20.6	28.2	33.5	34.0
Mustard gas	21.8	19.8	25.7	31.9	32.9
Water	15.9	14.1	18.9	23.0	
Amyl chloride			18.0	22.6	
Benzene	15.5	13.6	17.3	22.0	
Methyl alcohol	13.5	12.0	15.7	19.5	
n-Hexane			13.3	16.8	

TABLE V

ISOPIESTIC RATIOS FOR VARIOUS VAPORS

Vapor	Ratios to No. 7 for charcoal No.:			
	1	2	3	9
Carbon tetrachloride	0.686	0.638	0.790	1.030
Phosgene	0.725	0.615	0.842	1.015
Mustard gas	0.683	0.621	0.805	1.032
Water	0.692	0.613	0.823	
Amyl chloride			0.797	
Benzene	0.705	0.618	0.787	
Methyl alcohol	0.692	0.615	0.805	
n-Hexane			0.792	
Average	0.697	0.620	0.805	1.026
Supplier's *V.A.* ratios	0.620	0.643	0.836	1.107

vapors appear to be the same for each charcoal sample, taking into consideration the nonuniformity of the charcoal, so that any one of the liquids could serve as a medium for the activity test at saturation.

To illustrate the latter point further, the specific gravities of the various liquids used were measured at room temperature (24° to 25° C.) and the weights of adsorbed material converted into volumes. These values are given in Table VI.

With the exception of water, the liquid volumes are very uniform for each charcoal sample and show agreement with the rule given by McBain (6): "Equal volumes of different vapours, measured in the form of the corresponding liquids are sorbed by a given charcoal when P is approaching P_s". Water, however, gives a lower volume in each case. This indicates that the simple picture of capillary condensation cannot be used to explain this phenomenon.

TABLE VI

LIQUID VOLUMES ADSORBED

Vapor	Liquid specific gravity	Cc. of corresponding liquid adsorbed per 100 cc. of charcoal No.:				
		1	2	3	7	9
Carbon tetrachloride	1.58	16.6	15.4	19.1	24.2	24.9
Phosgene	(1.39)*	17.5	14.8	20.3	24.1	24.5
Mustard gas	1.27	17.2	15.6	20.1	25.1	25.9
Water	1.00	15.9	14.1	18.9	23.0	
Amyl chloride	0.880			20.4	25.7	
Benzene	0.876	17.7	15.5	19.8	25.1	
Methyl alcohol	0.792	17.1	15.2	19.8	24.6	
n-Hexane	0.660			20.2	25.4	
Average		17.0	15.1	19.8	24.6	25.1

* *International Critical Tables* (4).

Acknowledgments

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n-EICOS-11-ENOIC ACID[1]

By C. Y. Hopkins, Mary J. Chisholm, and John Harris

Abstract

Natural sources of *cis-n*-eicos-11-enoic acid, a convenient method of isolating the acid, and its conversion to the *trans*-form are described. It was found that the principal eicosenoic acid of a sample of Atlantic cod liver oil (*Gadus callarias*) was the $\triangle^{11}$ isomer, in contrast to the reports of other workers who investigated Pacific codfish and found only the $\triangle^{9}$ isomer. The following derivatives are reported: *cis-n*-eicos-11-enoic anilide, N-hydroxyamide, epoxide, chlorohydrin; 11,12-dihydroxyeicosanoic acid (low-melting isomer), and its amide; *trans-n*-eicos-11-enoic acid, its hydrazide, and N-hydroxyamide. Some new physical constants of the *cis*-acid and its methyl ester are given.

Relatively little is known of the eicosenoic acids, $C_{20}H_{38}O_2$, although the neighboring acids of the even-numbered series, oleic and erucic, have been studied in considerable detail. Discovery of good sources of *cis-n*-eicos-11-enoic acid provided an opportunity for further investigation of this substance and its derivatives.*

Eicosenoic acid has been isolated from a number of marine animal oils and fish oils, especially cod liver oil (3), and from jojoba seed (7, 13), hare's-ear mustard seed (12), and rapeseed (6). A small amount of the acid has been found in hog fat (16) and some evidence of its presence has been noted in various seed fats such as those of *Nephelium lappaceum* L. (11), *Erythrina Crista-Galli* L. (4), and *Lophira alata* Banks (15). The $\triangle^{11}$ isomer has been prepared artificially by degradation of erucic acid (2) and by condensation of oleyl bromide with malonic ester (22).

Two forms of eicosenoic acid have been reported in nature: $\triangle^{9}$ and $\triangle^{11}$. Eicosenoic acid from plant sources so far examined has been shown to be eicos-11-enoic acid (7, 12). The acid from hog fat was the same (16).

Among the oils of marine fauna, the $\triangle^{11}$ isomer has been reported in menhaden body oil by Baldwin and Parks (1) and in cod liver oil by Vesely and Chudozilov (22).

The usual form in marine oils, according to Japanese workers, is eicos-9-enoic acid. Evidence for the $\triangle^{9}$ structure was obtained by ozonolysis experiments (18, 21). Takano and Toyama and coworkers concluded thereby that cod liver, Japanese sardine, and herring oils contained eicos-9-enoic acid. Thus there is disagreement as to whether the eicosenoic acid of cod liver oil has the $\triangle^{9}$ or $\triangle^{11}$ structure.

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* *The natural acid is presumed to be the cis-form by analogy with oleic acid, i.e., assuming that the lower melting form has the cis-configuration.*

Vesely and Chudozilov prepared *cis*- and *trans*-eicos-11-enoic acid from oleyl bromide by condensation with malonic ester. They compared derivatives of the *cis*-acid with those of natural eicosenoic acid from cod liver oil and concluded that they were identical.

Results and Discussion

Specimens of eicosenoic acid were prepared during the present investigation from jojoba seed (*Simmondsia californica* Nuttall), hare's-ear mustard seed (*Conringia orientalis* L.), and cod liver oil (Atlantic codfish, *Gadus callarias*).

Jojoba seed is the most convenient source. Care must be taken to remove completely the long-chain alcohols which are not easy to separate from the fatty acids by the ordinary procedures. A method is described in the experimental part.

It has been shown by oxidation with permanganate in acetone that the eicosenoic acid of jojoba seed has the $\triangle^{11}$ structure (7). Similarly, it has been proved by periodate oxidation that the acid from *Conringia orientalis* L. is the $\triangle^{11}$ form (12).

In the present work, a very careful purification of eicosenoic acid from cod liver oil was made and the product was compared with pure eicos-11-enoic acid from jojoba seed by means of suitable derivatives. The results provide conclusive evidence that our specimen of eicosenoic acid from cod liver oil is eicos-11-enoic acid (Table I).

TABLE I

MELTING POINT OF DERIVATIVES, °C.

Substance	From cod liver oil	From jojoba seed	Mixed
11,12-Dihydroxyeicosanoic acid (by H_2O_2)	94–95.5	95–96	94.5–96
11,12-Dihydroxyeicosanoic acid (by $KMnO_4$)	129–130	129–130	129–130
cis-Eicos-11-enoic acid N-hydroxyamide	67–68	69–70	68–69

The two isomeric dihydroxy derivatives of the acid are analogous to the two dihydroxystearic acids prepared from oleic acid in similar manner.

For further proof of structure, the dihydroxyeicosanoic acid from cod liver oil, m.p. 94° to 95.5° C., was subjected to periodate oxidation and the products were identified as 10-aldehydo-decanoic acid and nonaldehyde (Table II). The original substance must therefore be *n*-eicos-11-enoic acid.

Derivatives of the cod liver oil acid were a little more difficult to purify than those of the jojoba acid.

TABLE II

MELTING POINT OF CLEAVAGE PRODUCTS, °C.

Substance	From cod liver oil	From jojoba seed	Mixed
10-Aldehydo-decanoic acid semicarbazone	161–162*	161–162	161–162
Nonaldehyde semicarbazone	94–95	96–97	95–97

** Mixed melting points with 8-aldehydo-octoic acid semicarbazone and with 12-aldehydo-dodecanoic acid semicarbazone showed a depression of about 10° C. in each case.*

The occurrence of eicosenoic acids in marine fauna is summarized in Table III.

TABLE III

EICOSENOIC ACIDS IN MARINE FAUNA

Source	Reference	Isomer reported	M.p. of acid, °C.	M.p. of dihydroxy derivative (by $KMnO_4$), °C.
Cod liver oil ("Lofoden")	3	—	24.5	127.5 to 128
Cod liver oil	22	Δ^{11}	23 to 24	—
Cod liver oil	21	Δ^{9}	24 to 24.5	—
Japanese sardine	18	Δ^{9}	22 to 23	—
Japanese sardine	21	Δ^{9}	24 to 24.5	128 to 128.5
Pilot whale (*Globiocephalus sieboldii* Gray)	20	—	23.5 to 24	126.5 to 127*
Sperm whale	10	—	—	—
Menhaden (*Brevoortia tyrannus*)	1	Δ^{11}	—	115.5 to 116**
Menhaden (*Brevoortia tyrannus*)	17	—	—	129.2 to 129.5
Cod liver oil (*Gadus callarias*)	Present work	Δ^{11}	20.5	129 to 130

** Marked lowering of melting point reported with 9,10-dihydroxy acid.*
*** Admittedly impure.*

A carefully purified sample of *cis*-eicos-11-enoic acid from jojoba seed (m.p. 22° C.) had refractive index $n_D^{20°} = 1.4616$, $n_D^{25°} = 1.4600$.

Purified samples of methyl *cis*-eicos-11-enoate from jojoba seed and from cod liver oil had refractive index $n_D^{20°} = 1.4550$; $n_D^{25°} = 1.4531$. The ester melts at about $-15°$ C.

Few derivatives of eicos-11-enoic acid are known. Derivatives prepared during the present work are listed in Table IV.

TABLE IV

DERIVATIVES OF *n*-EICOS-11-ENOIC ACID

Compound	M.p., °C.	Formula	Analysis, %
cis-Eicos-11-enoic amide	79–80*	$C_{20}H_{39}ON$	Found: N, 4.5, 4.4 Calc.: N, 4.5
cis-Eicos-11-enoic anilide	51–52	$C_{26}H_{43}ON$	Found: N, 3.5, 3.5 Calc.: N, 3.6
cis-Eicos-11-enoic N-hydroxy-amide	69–70	$C_{20}H_{39}O_2N$	Found: N, 4.5 Calc.: N, 4.3
11,12-Epoxyeicosanoic acid	55.0–55.5	$C_{20}H_{38}O_3$	Found: C, 73.2; H, 11.4 Calc.: C, 73.6; H, 11.7
11,12-Dihydroxyeicosanoic acid, low m.p. isomer	95–96	$C_{20}H_{40}O_4$	Found: C, 69.8; H, 11.5 Calc.: C, 69.7; H, 11.7
11,12-Dihydroxyeicosanoic amide, low m.p. isomer	116–117	$C_{20}H_{41}O_3N$	Found: N, 4.1, 4.2 Calc.: N, 4.1
Chlorohydrin of eicos-11-enoic acid	39–40	$C_{20}H_{39}O_3Cl$	Found: Cl, 9.92 Calc.: Cl, 9.77
trans-Eicos-11-enoic acid	52–53**	$C_{20}H_{38}O_2$	Found: Equiv. 311, 312 Calc.: Equiv. 310.5
trans-Eicos-11-enoic N-hyd-roxyamide	93–94	$C_{20}H_{39}O_2N$	Found: N, 4.2, 4.4 Calc.: N, 4.3
trans-Eicos-11-enoic hydrazide	103	$C_{20}H_{40}ON_2$	Found: N, 8.4, 8.4 Calc.: N, 8.6

* *Vesely, 78° to 79° C.* (22).
** *Vesely,* 53° to 54° C.

trans-Eicos-11-enoic acid has previously been prepared by partial synthesis. Bodenstein reported a melting point of 50° C. (2) and Vesely and Chudozilov found it to be 53° to 54° C. (22).

The eicosenoic acid from Japanese sardine oil (reported to be the $\triangle^9$ isomer), when converted to the *trans*-form, melted at 51.5 to 52° C. (21).

Experimental

Isolation of Eicosenoic Acid from Jojoba Seed

A quantity of *cis*-eicos-11-enoic acid was isolated from jojoba seeds.* The procedure differed from that of Green, Hilditch, and Stainsby in the method of removing the long-chain alcohols.

The liquid wax (350 gm.) was saponified with ethanolic potassium hydroxide, and after distilling off most of the ethanol, the mixture was dried on the steam bath to a paste. The paste was leached with several portions of

* *Kindly furnished by Mr. Frederick Gibson.*

acetone and the undissolved soaps were extracted further with acetone in the Soxhlet. Finally the acetone was replaced by ether and the extraction was continued for several hours.

The residual soaps were boiled with excess 1:1 hydrochloric acid in the presence of chloroform. The chloroform layer was separated off. It yielded the free fatty acids upon removal of the solvent.

The mixed fatty acids were converted to methyl esters and these were fractionally distilled in the usual way. The three main fractions had iodine values 79.1, 78.1, 77.4 respectively (methyl eicosenoate: 78.4). It is estimated from the fractionation data that eicosenoic acid constitutes at least 60% of the total fatty acids of the seed and that the content of docosenoic acid is not more than 25%. These figures correspond fairly well with those of McKinney and Jamieson (13).

The methyl eicosenoate was recrystallized from acetone (10 ml. per gm.) at −40° C. The product had iodine number 78.4 (theory 78.4). A portion was hydrolyzed to the acid and the acid was recrystallized from acetone at −15° C. It had iodine number 81.4 (theory, 81.8). The melting point was 22° C. when determined by the open capillary tube method described by Hilditch (9). The setting point (titer) was 21° to 22° C.

Isolation from Cod Liver Oil

A specimen of cod liver oil from the species *Gadus callarias* (North Atlantic) was kindly supplied by Dr. S. A. Beatty. The oil (iodine number, 156) was converted to methyl esters in 600 gm. lots and these were fractionally distilled in the usual way at a pressure of less than 1 mm. The distillation curve showed flattened portions corresponding to the boiling points of C_{16}, C_{18}, and C_{20} esters. The C_{20} fraction was subjected to fractional crystallization from acetone at low temperatures (10 ml. per gm.). The product, methyl eicosenoate, had iodine number 78.7 (theory, 78.4). The free acid obtained therefrom melted at 20.5° C. The compounds listed in Tables I and II were prepared from this acid.

Isolation of the acid from hare's-ear mustard seed (*Conringia orientalis* L.) has already been described (12).

The acid obtained from jojoba seed was used for the preparation of derivatives listed in Table IV.

cis-Eicos-11-enoic Amide and Anilide

The acid chloride was readily prepared by allowing the free acid to stand overnight with phosphorus trichloride. On treating the acid chloride with ammonia, the amide was precipitated. It was recrystallized from ethyl acetate. The anilide, prepared in the same way, was recrystallized from acetone.

cis-Eicos-11-enoic N-Hydroxyamide

Methyl eicosenoate and hydroxylamine were reacted in alkaline solution according to the general method of Renfrow and Hauser (14). The potassium salt, which precipitated slowly, was filtered off and acidified cautiously. The N-hydroxyamide was recrystallized several times from acetone.

11,12-Epoxyeicosanoic Acid

cis-Eicos-11-enoic acid was converted to the epoxy compound by the general method of Findley, Swern, and Scanlan (5) using peracetic acid. The product was recrystallized successively from petroleum ether, acetone, and methanol. It is slightly soluble in petroleum ether but readily soluble in ethanol, benzene, and chloroform.

11(or 12)-Chloro-12(or 11)-hydroxyeicosanoic Acid

The epoxy acid was treated with dry hydrogen chloride in dry ether. After four crystallizations from petroleum ether the product melted at 39° to 40°. It is readily soluble in ethanol, benzene, acetone, and chloroform.

11,12-Dihydroxyeicosanoic Acid

The low-melting isomer, prepared by the action of hydrogen peroxide on eicosenoic acid, was prepared earlier (12). The melting point and analysis are given in Table IV. The high-melting isomer, m.p. 130° C., is well known.

11,12-Dihydroxyeicosanoic Amide

The low-melting isomer was prepared by the action of hydrogen peroxide on eicosenoic amide in the presence of formic acid. The product was recrystallized from ethyl acetate.

trans-Eicos-11-enoic acid

Isomerization of the *cis*- to the *trans*-acid was effected readily by means of Poutet's reagent, using the procedure of Griffiths and Hilditch (8) as follows.

One part of the reagent (6 gm. mercury dissolved in 15.6 ml. nitric acid, *d* 1.42) is added to 12 parts of the eicosenoic acid and the mixture is immediately cooled under the tap with vigorous shaking. It is allowed to stand for several hours and is then recrystallized from ethanol.

trans-Eicos-11-enoic N-Hydroxyamide

A specimen of *trans*-eicos-11-enoic acid was esterified with methanol via the acid chloride. The ester was treated with hydroxylamine by the procedure of Renfrow and Hauser (14) and the product was recrystallized twice from ethanol. It appeared to contain some dihydroxy amide. The desired product was obtained by warming with toluene, in which it is soluble, and filtering off the impurity. The N-hydroxyamide was isolated as lustrous white flakes, somewhat soluble in toluene and ethanol, but insoluble in water.

trans-Eicos-11-enoic Hydrazide

Methyl *trans*-eicos-11-enoate (3 gm.) was mixed with 3 ml. of hydrazine hydrate (85% solution) in 30 ml. of absolute ethanol. The mixture was refluxed on the steam bath for two hours, cooled in ice, and the crystalline product was filtered off. It was purified by washing with hexane and recrystallizing three times from toluene.

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